DIGESTION, STORAGE, AND TRANSLOCATION OF NUTRIENTS IN THE PURPLE SEA URCHIN (STRONGYLOCENTROTUS PURPURATUS)[1]

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The internal transport of nutrients in echinoderms has been a matter of interest since 1809 when the French Institute offered a prize for a description of the "circulatory" system of asteroids, echinoids, and the holothuroids (Tiedemann, 1816). Since that time the anatomy of various echinoderms has been studied by numerous investigators, prominent among whom were Perrier, Hamann, and Cuénot. The accumulated knowledge of the Phylum Echinodermata was presented in a treatise by Hyman in 1955.

A survey of this literature indicates a general recognition of three fluid systems, *i.e.*, the perivisceral fluid, the water vascular system, and the haemal system. However, the roles of these systems in the translocation of food remain obscure.

The vessels of the water vascular system and the sinuses of the haemal system are very narrow and delicate. Sampling of the fluids that they contain is extremely difficult. Therefore, examination of transport in these systems has been limited to microscopic observations of the movement of objects within the vessels or sinuses, particles of injected dyes or the naturally present coelomocytes being the objects observed (Perrier, 1875; Kawamoto, 1927; see also Hyman, 1955). The perivisceral fluid, which bathes the internal organs, is relatively large in quantity and more accessible to sampling and subsequent examination. This fluid from representatives of the more conspicuous classes, namely the holothuroids, asteroids, and echinoids, has been subjected to physiological and biochemical analysis (Jacobsen and Millott, 1953; Lasker and Giese, 1954; Boolootian and Giese, 1958; Farmanfarmaian, 1959; see also Hyman, 1955). Some observations in these studies have resulted in the assignment of various possible functions to the variety of coelomocytes which are to be found in this fluid as well as in the tissues of the animals (Hyman, 1955; Stott, 1955; Boolootian and Giese, 1958).

The suggestion that these cells may be involved in nutrient transport is based on very little experimental evidence. Phagocytosis of foreign materials such as carbon, carmine, and fat particles and their deposition in certain tissues may or may not simulate aspects of natural nutrient transport. Since phagocytic cells are known to ingest such non-nourishing and inert particles as polystyrene latex spherules with subsequent migration *in vitro* (Sbarra and Karnovsky, 1959, 1960), no *a priori* significance may be attached to such processes when they are observed *in vivo*. Even studies involving the injection of materials such as sugars or iron saccharate (Lasker and Giese, 1954; Stott, 1955), though useful under special

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circumstances, cannot be regarded as good indicators of the natural process of transport following the digestion and absorption of food. The considerations mentioned above indicate the desirability of a systematic study of the translocation of nutrients following natural absorption. Knowledge of this phenomenon is prerequisite to the understanding of much of the biology of echinoderms. It has been possible to carry out such a study through the feeding of algae labelled with carbon-fourteen to sea urchins.

Materials and Methods

The purple sea urchin, *Strongylocentrotus purpuratus,* was chosen for these investigations. Animals with a test diameter of 4–7 cm. were starved for five to eight weeks prior to experimental feedings. The animals were maintained in well-aerated sea water at 15° C. in the laboratory.

The red alga, *Iridaea flaccidum,* was used as food in the experiments. (The name *Iridophycus* has been used to describe this genus also.) This material was chosen as food because it is abundant in the habitat of the urchin throughout the year; it constitutes one of the animal's natural foods as judged by the examination of gut contents in the field; and it is capable of maintaining urchins in good condition for over a year in laboratory aquaria when used as the sole source of food. Much of the biochemistry of this alga is known (Hassid, 1936; Bean *et al.,* 1953; Bean and Hassid, 1955).

Cut discs of the alga were labelled with C^{14} according to the method of Bean *et al.* (1953), using the gas-sealed apparatus, which they describe, as a photosynthesizing chamber. One to three grams of labelled alga could be prepared in this manner. Amounts of the alga less than one gram were labelled in sealed vials in a sea water solution of $NaHC^{14}O_3$ at pH 8. In either case the amount of $C^{14}O_2$ available to the alga was empirically adjusted to give $2–4 \times 10^4$ counts per minute/mg. wet weight of alga. The photosynthetic assimilation of $C^{14}O_2$ was allowed to proceed for 10 hours at about 18° C. The algal discs were sampled in several places to determine their specific activity. The discs were then drained and weighed carefully before being fed to the animals.

The animals were fed in sealed jars maintained at 15° C.; see Figure 1. These jars were provided with capillary air inlets which opened below the sea water and vacuum outlets from the gas phase. It was thus possible to draw off metabolic $C^{14}O_2$ into a $Ba(OH)_2$ trap and provide the animal with continuous aeration without contaminating the laboratory. The animals were never allowed more than five hours of feeding time. After the desired period of feeding, the remainder of the algal disc was removed and weighed as before.

All of the tissues were freshly sampled in such a manner as not to contaminate one another. This was achieved by careful dissection, the use of several sea water washes, and frequent changes of dissecting instruments. Duplicate samples were taken in all cases. When the final specific activity of duplicate samples differed by more than 10%, the samples were rejected. Rejection occurred most frequently when the gonads were ripe and spawning took place during sampling. Other sources of error responsible for large differences in duplicates were the difficulty of weighing fresh tissue to constant weight and contamination during dissection.

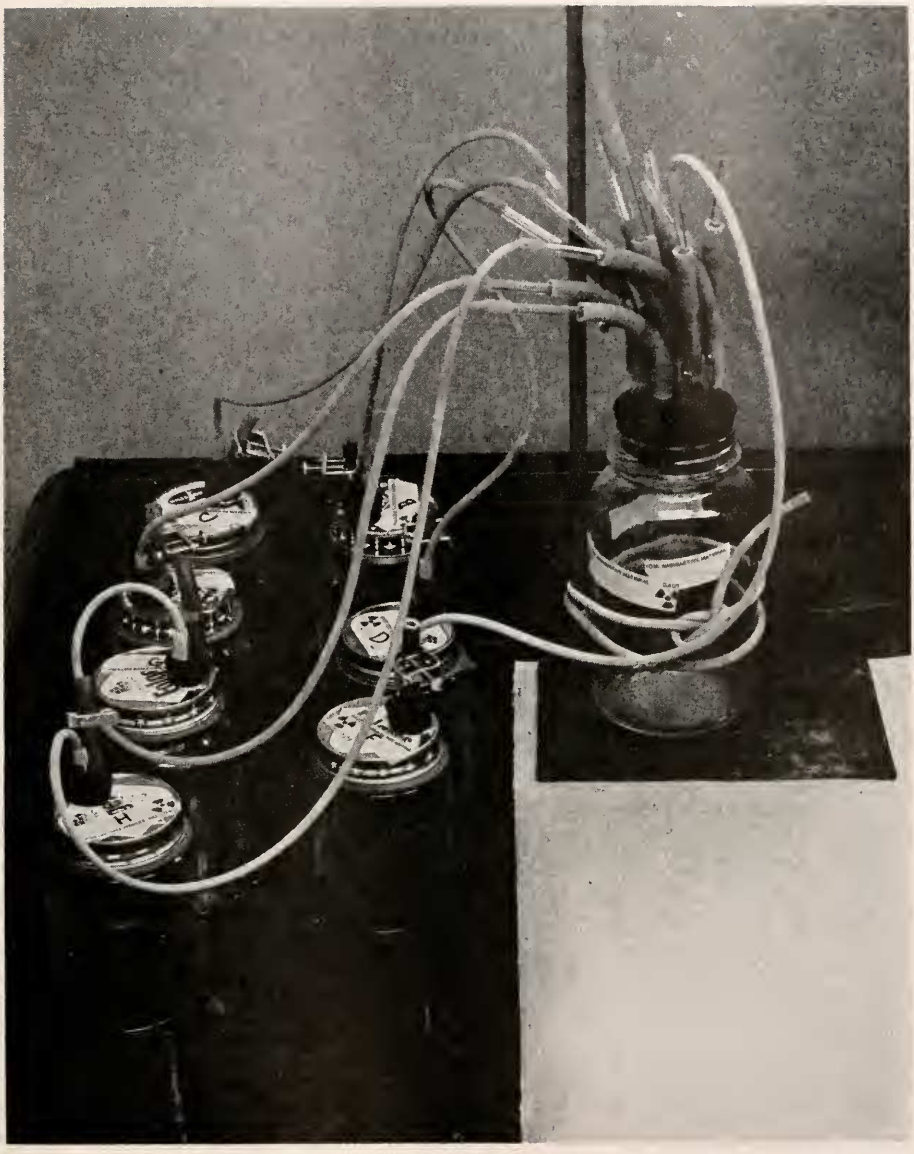

FIGURE 1. Arrangement of feeding jars in which sea urchins were fed radioactive algae.

The perivisceral fluid was sampled by a one-milliliter syringe through the peristomeal membrane and directly from the main coelom. A volume of 10% solution of ethylene diamine tetraacetic acid at pH 8.0 equal to the sample volume was used as anticoagulant. The cells and plasma were separated by centrifugation as desired.

Since the specific activities of various tissues were to be compared, it was necessary to use a homogeneous suspension or solution of the tissues. A high

degree of homogeneity was attained by digesting samples of tissue in NaOH with the aid of 30% H_2O_2 and heat. Soft animal tissue samples of 10–20 mg. and 100–200 mg. samples of test wall were placed in graduated centrifuge tubes and covered to the 0.5-ml. mark with 1 *M* NaOH in sea water. Algal samples were covered with 10 *M* NaOH during the digestion and subsequently diluted with sea water in a manner calculated to keep the concentration of salts uniform. Time of digestion and the amount of H_2O_2 used were as needed. Unless otherwise stated, the activity of the perivisceral fluid was determined without digestion.

Samples of digests were placed on stainless steel planchets, dried, and counted in a Nuclear Chicago gas-flow counter, model D-47 with mica window, equipped with scaling unit model 161A, sample changer model c-110B, and printing timer model c-111B. All errors in the counting procedure were corrected by the methods of Calvin *et al.* (1949) and Kamen (1957). Particular attention was paid to errors in geometry and absorption because of the high salt content of the samples.

Paper chromatograms were analyzed by an Actigraph model c-100A combined with scaler model 1620A and recorder R1000.

Results and Discussion

Digestion. The digestive tube of the purple sea urchin consists of a buccal pouch, a short pharynx, and an esophagus that enters the first convolution of the tube. This portion of the tube immediately below the esophagus will be referred to as the stomach. The stomach opens into the second convolution, referred to here as the intestine, and the latter terminates in a short rectum which opens to the outside via an anus. The stomach and intestine each have five festoons which will be designated one to five in sequence from mouth to anus.

The conversion of algal C^{14} into animal C^{14} was determined in the following manner: the specific activity of the alga pieces fed and the specific activity of the contents of the digestive tubes of animals sacrificed at various times were determined. These results are presented in Table I, and show that conversion efficiency, as is indicated by efficiency of digestion and absorption, is about 90% when the animal is fed a limited, 20 to 100 mg. wet weight, amount of *Iridaea*. Since the

Table I

Conversion efficiency of C^{14}-labelled substances from Iridaea *tissue into sea urchin tissue*

Days after start of feeding	C/M/mg. of dry digest of		% Activity incorporated	Nature of gut content
	Algae fed	Gut content		
1	526	38.6	93	Bite form No bags No bacterial enrichment
2	490	50.3	90	As above
3	555	36	93	Bag form Bacterial enrichment
4	641	53	91	Bag form High bacterial enrichment
9	294	33	89	As above

digestive efficiency remains nearly the same throughout the interval of one to nine days after a limited amount of food is ingested, the results suggest that digestion and absorption of at least the labelled portion of the food occurs primarily on the first day, and thereafter the remnants of food are on their way to defecation. Since the food has not passed the fourth festoon of the stomach by the second day, the esophagus, the stomach, or both would appear to be the main site of digestion in the animal. The algal material in the first four festoons of the stomach is essentially in bite form and free of bacterial enrichment. As is indicated in Table I, bacteria become conspicuous by the third day when the material has passed the fourth and fifth festoons of the stomach. At this point the material has been converted from the bite form to the bag form. These different kinds of gut contents are pictured in Figure 2. These observations suggest that the digestion of *Iridaea* in

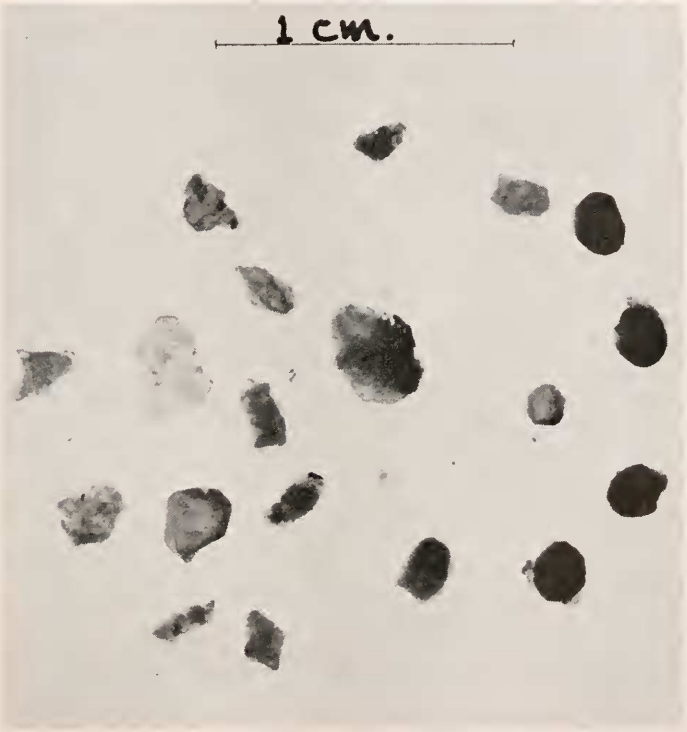

FIGURE 2. Pieces of *Iridaea* removed from various parts of the digestive tube. The transition from bite form from the stomach to bag form from the intestine is seen from left to right.

the stomach is not dependent on bacterial action and is more likely under the influence of digestive enzymes secreted by the sea urchin.

This suggestion was confirmed by examining the digestive ability of extracts of esophagus, stomach, and intestine. The tissues, washed free of gut contents, were homogenized in sea water and freed of large tissue fragments by centrifugation. Washed soaked agar and washed bite-sized pieces of *Iridaea* were used as substrates. Toluene was added to prevent bacterial growth. Reducing sugar was

TABLE II

*Reducing sugar liberated by tissue extracts**

	Agar	*Iridaea*
Extract of esophagus	76 μg†	60 μg
Extract of stomach	160	80
Extract of intestine	80	40
Pooled extract	240	80
No extract	56	40

* Twelve-hour incubation at 15° C.; 50 mg. of agar and *Iridaea* used as substrates. The amounts of tissue extract of esophagus, stomach, and intestine were 5, 71, and 78 μg protein. The pooled extract was a preparation containing equivalent amounts of each of the extracts. All values were corrected for the contribution of reducing material in the extracts.

† Values equivalent to μg glucose.

determined after incubation by the method of Park and Johnson (1949). Table II shows the results and conditions of incubation. It should be noted that some reducing material is solubilized from *Iridaea* and agar under the conditions of incubation. However, larger amounts appeared in solution after exposure to extracts of stomach or esophagus. The extract of intestine when tested by itself did not liberate additional reducing material from *Iridaea*. Whether or not it contributes to the material released by the extract pool remains to be determined. Both the stomach and the esophagus appear to possess digestive enzymes. Since the amount of material in the extract of esophagus is only one-fifteenth the amount in the other two extracts, the results suggest that the esophagus may contain appreciable amounts of digestive enzymes. The presence of enzymes capable of hydrolyzing agar is definitely indicated.

Lasker and Giese (1954) reported the presence of enzymes in extracts prepared from the whole digestive tube of the purple sea urchin. Enzymes capable of digesting casein, starch, and iridophycin, a galactan prepared by Hassid from *Iridaea,* were detected. Eppley and Lasker (1959) have demonstrated alginase and algin depolymerase activity in the digestive tract of this animal. The failure of Lasker and Giese (1954) to detect agar-digesting enzymes may have been due to their use of agar warmed to 37–40° C. Since this temperature is more than 10 degrees above the lethal temperature of the animal, it may result in inactivation of the enzymes responsible for agar digestion.

While the Aristotle's lantern of the sea urchin is a magnificent masticatory apparatus that is capable of reducing the alga to small pieces before it reaches the esophagus, the final disintegration of algal structure by the subsequent action of digestive enzymes has not been observed. There is no question that the bacteria from the intestine of the sea urchin can attack algae, but their role in the digestion of that part of the food which is normally assimilated may not be significant. When *Iridaea* is made available to the sea urchin in plentiful quantities, the animal often feeds continuously and defecates rapidly. Under these conditions the feces are usually in bite form and without bacterial enrichment. The disintegration of algal structure observed in the distal festoons of the intestine when defecation is delayed may provide additional assimilable material. The uptake of such material by the animal would not have been observed in the experiments reported here since labelling of the structural elements of the algal cells would have to have been ac-

complished during their growth. However, the lack of participation of microorganisms in the digestion of the labelled portion of the alga is further indicated by studies in which bacterial action was inhibited by antibiotics. C^{14}-labelled algal material kept in strong solutions of streptomycin and penicillin prior to feeding gave the same digestive efficiency and no bacterial enrichment of the feces.

That the microorganisms observed in feces do not constitute forms unique to the urchin is suggested by the following observations: finely chopped *Iridaea* in sterile sea water was incubated for four days in the dark at 15° C. Similar preparations were inoculated with bacteria-enriched contents of the rectum. Both series yielded a grossly similar collection of bacteria and protozoa. These preliminary experiments suggest that the fauna and flora of the sea urchin gut may represent the symbionts of the ingested alga. Mastication and digestion by the sea urchin render the material more susceptible to attack by these microorganisms and results in the bacterial and protozoan enrichment so often observed in the intestine of the animal.

Storage of digested food. From studies on the reproductive physiology and biochemistry of the purple sea urchin, the site of food storage has become a point of controversy (Giese *et al.*, 1958). It was, therefore, desirable to determine the site of deposition of C^{14}-labelled compounds in the animal. The overall specific activity of the animal was calculated in the following manner:

$$\text{Specific Activity of animal} = \frac{(\text{total activity of ingested algal material})\ (\text{conversion efficiency})}{(\text{total wet weight of the animal in mg.})}$$

The specific activities of various tissues of the sea urchin were determined and expressed in relation to the overall activity of the animal, the latter being given a value of 1.00. These results are presented in Table III. The esophagus and the first festoon of the stomach appear to be the main sites of nutrient storage. The drop in activity of the walls of the digestive tract by the seventh day after feeding was not accompanied by a general shift of material to all other tissues. It is well

TABLE III

Specific activity of various tissues relative to the calculated overall specific activity of the animal in counts/minute/mg. wet weight

Days after feeding	1	2	3	7
Tissue				
Whole animal	1.0	1.0	1.0	1.0
Body wall				
interambulacral	0.1	0.3	0.1	0.3
ambulacral	0.3	0.5	0.2	0.5
Gonad	1.6	1.0	0.8	1.3
Esophagus	20.4	60.0	26.3	21.2
Stomach				
Festoon 1	37.5	86.0	47.0	27.3
Festoon 4	7.9	14.5	5.2	11.8
Intestine				
Festoon 1	6.2	15.5	3.5	10.5
Festoon 4	2.3	12.0	1.6	4.7

established that a starving sea urchin will resorb its gonads, but there is no indication that the gonads constitute the natural storage organ of these animals. A recent study of Giese (1961) indicates that lipid is the main reserve food of *Strongylocentrotus purpuratus, S. franciscanus,* and *Allocentrotus fragilis.* The lipid is stored in the wall of the intestinal tract and is observed to decrease in amount during starvation. In the asteroids the hepatic caeca have been shown to be the organs of storage (Farmanfarmaian *et al.,* 1958; Anderson, 1953). Anatomically these are diverticula of the digestive tract, and the festoons of the sea urchin digestive tract may be compared to them in function.

Translocation of Nutrients

1. *The haemal system*

In general this system consists of poorly defined sinuses often filled with red coelomocytes. No movement of fluid within any part of the system has been observed even though a rhythmic beat may be seen in the outer sinus of the stomach and its collateral sinuses. In spite of careful attempts, the fluid could not be sampled without contamination. Therefore, the role of the haemal system in the transport of nutrients was assessed by indirect means. C^{14}-labelled material appears in the gonads even on the first day after feeding; see Table III. Each gonad

Figure 3. The aboral haemal ring and its short sinuses which penetrate the gonads. The arrow indicates one of the sinuses. Photograph is of a fresh specimen enlarged two times.

is penetrated by only one sinus from the aboral haemal ring. Therefore, it was possible to sever this sinus and compare the specific activity of a gonad thus isolated from the haemal system with its normal neighbor; see Figure 3. This delicate operation was accomplished by carefully drilling a half-millimeter hole just oral to the gonopore in the center line of the interambulacral region. Under a dissecting microscope equipped with a strong spotlight, the haemal sinus and the gonoduct of the gonad were seen just inside the test wall. These tubes could then be gently lifted up by means of a finely bent needle and severed by a microscalpel made from a piece of razor blade. The hole was closed with a fine wooden plug covered with Vaseline. The operation did no apparent harm to the animal nor altered its behavior in any observable manner. The cut tubes constricted and healed within 24 hours, and the connection between the aboral haemal ring and the gonad began to regenerate by about the tenth day after the operation.

Twenty-four hours after the operation, animals were fed C^{14}-labelled *Iridaea*, and the specific activity of the gonads was determined at intervals following feeding. The results are presented in Table IV. In all cases the isolated gonad and its

TABLE IV

Comparison of the specific activity of normal gonad with neighboring gonad whose connection to the aboral haemal ring was experimentally severed

Days after feeding	Sex	Gonad gravidity	Animal wet weight in grams	C/M/mg. wet weight		
				Overall animal	Gonad experimental	Gonad control
1	♀	Ripe	31	160	275	253
2	♀	Ripe	32	24	26	25
3	♂	Ripe	25	230	177	181
7	♂	Ripe	25	42	54	55

neighbor contained essentially the same amount of activity, irrespective of sex or number of days after the start of feeding. The active materials, therefore, must have arrived via routes other than the haemal system.

These experiments were carried out on animals with ripe gonads. The possibility that the haemal system plays a special role in nutrient transport to the gonads during their period of buildup, *i.e.*, August through November (Giese *et al.*, 1958), must be examined by experimentation. However, data presented in a later section indicate the more general route of nutrient transport.

The function and microanatomy of the haemal system remain an enigma. This collection of sinuses does not appear to constitute a circuitous system, nor does it appear to have, functionally speaking, a point of origin or terminus. The enigma is no less striking when other classes of the phylum are considered (Hyman, 1955). The haemal system may be the vestige of a true transport system in the ontogeny or phylogeny of echinoderms.

2. *The water vascular system*

This system consists of a well-defined water vascular ring, the stone canal, and the five radial canals that penetrate the ambulacral regions through the auricles and

thence send side branches to ampullae of the podia. Although coelomocytes may be observed to move within the lumen of the radial canals, there is no clear direction of flow. There is good evidence to support the suggestion that the canals of the water vascular system maintain the hydrostatic pressure required for the operation of the podia and their ampullae (Cuénot, 1948; Hyman, 1955).

Because of the difficulty encountered in attempts to sample the fluid of this system, an indirect method similar to that used for the haemal system was adopted.

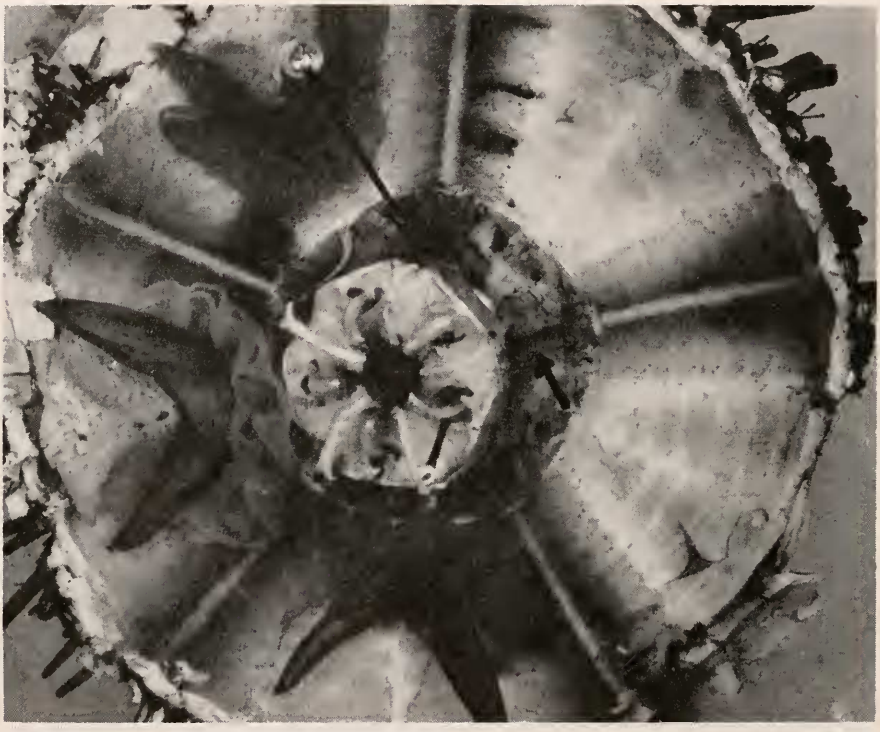

FIGURE 4. A view of the oral side of the animal from within. The lantern has been pushed to one side. The arrow points to the radial haemal sinus and the radial water canal just prior to their entrance into the orifice of the auricle. Photograph of fresh specimen twice enlarged.

The operation was considerably simpler because the radial haemal sinus and the radial water canal adhere firmly to the peristomeal membrane just prior to their entrance into the auricle. (See Fig. 4.) A 1-mm. incision through the soft peristomeal membrane across the center line of the ambulacral region exposed these tubes. The tubes were severed as described in the operation on the haemal system. Healing and regeneration are about the same as those described for the haemal system. Twenty-four hours after the operation, animals were fed C^{14}-labelled *Iridaea*, and the specific activity of the ambulacral areas determined in the usual manner. The results of these experiments are presented in Table V. No significant difference was noted between the ambulacral regions isolated from the radial haemal sinus and water canal and neighboring ambulacra which were left connected.

TABLE V

Comparison of the specific activity of normal ambulacrum with neighboring ambulacrum whose radial water canal and haemal sinus were experimentally severed at the auricle

Days after feeding	Animal wet weight in grams	C/M/mg. wet weight		
		Overall animal	Ambulacrum experimental	Ambulacrum control
1	31	160	53	55
2	32	24	14	13
3	29	28	13	12
7	25	42	22	22

It seems reasonable to conclude that the water vascular system does not play a significant role in the transport of digested food under these circumstances. These experiments provide additional evidence for the lack of importance of the haemal system in this process.

3. *The perivisceral fluid*

The perivisceral fluid occupies the main coelomic chamber of the sea urchin and is kept in circulation by the cilia of the epithelial lining of the coelom. All the internal organs of the animal are bathed by this fluid. Numerous cells, the coelomocytes, of seven different kinds may be observed in this fluid (Boolootian and Giese, 1958). The plasma phase of this fluid contains low levels of nitrogenous compounds, carbohydrates, and possibly fats (Giese *et al.*, 1958; see also Hyman, 1955) and has essentially the same salt composition as sea water.

Since there are several milliliters of this fluid in the main coelom which may be tapped by a syringe via the soft peristomeal membrane, it was possible to sample this fluid directly and determine the level of activity at various intervals after the start of feeding. Figure 5 shows the results of a series of such experiments. The labelled substances reach a peak level within six hours and then decrease in concentration to a fairly constant level within the first 24 hours. Thereafter the level is generally maintained for at least nine days, the period during which samples were usually taken and examined. The general shape of the graphs in Figure 5 was independent of animal size and quantity of C^{14}-containing material fed.

In studies on the respiration of the purple sea urchin, it was demonstrated that the perivisceral fluid serves as a medium for respiratory gas exchange (Farmanfarmaian, 1959). It has also been demonstrated that the oxygen uptake of a starved urchin may increase by as much as 50% following feeding (unpublished data). The possibility that the peak levels depicted in Figure 5 are due to a gush of respiratory $C^{14}O_2$ was tested in the following way: duplicate sets of samples were taken from an animal. One set was acidified to pH 2 in order to convert any $C^{14}O_2$ to a volatile form. After drying, the activity in both sets was determined. Figure 6 shows the result of this experiment. The activity found in the perivisceral fluid is not due to respiratory $C^{14}O_2$, which must be present at any given time at a negligible level.

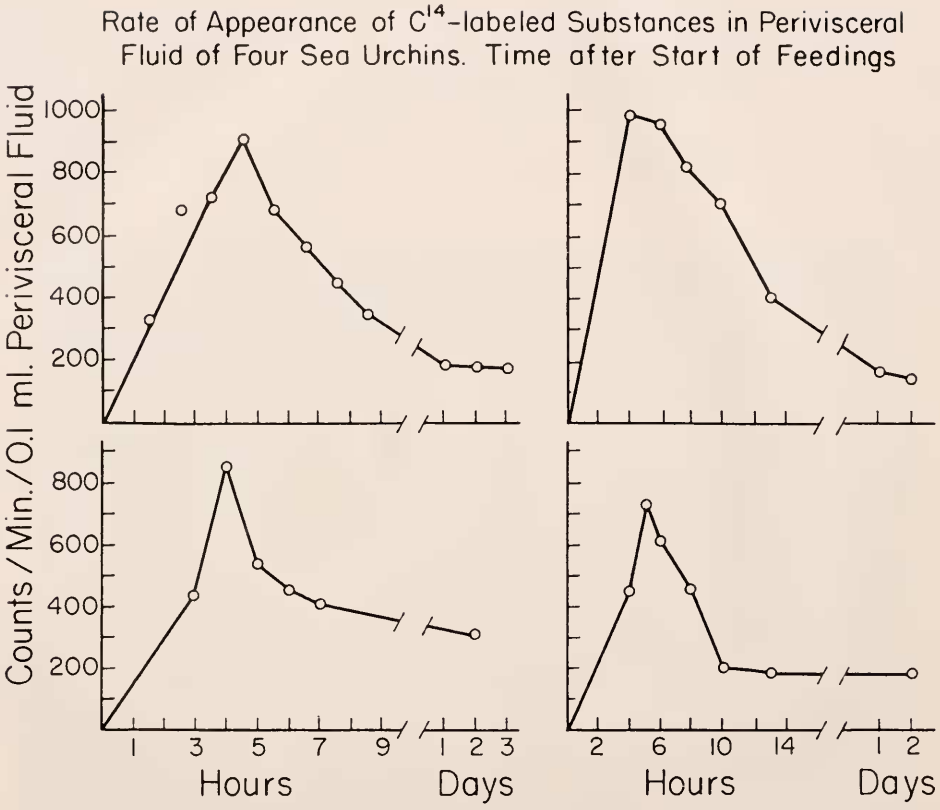

FIGURE 5.

The rise and fall in the activity of the perivisceral fluid suggested two hypotheses:

a. The transfer of nutrients from the digestive tube to the perivisceral fluid is controlled by some mechanism, *e.g.* neurosecretions. When a starved animal is fed, nutrients are rapidly mobilized, and a peak level of activity in the perivisceral fluid is observed. Within the first day after feeding, the tissues of the animal attain a state of relative sufficiency, and a feedback mechanism reduces and maintains the level of mobilized nutrients in a steady-state.

b. The peak level of activity in the perivisceral fluid is due to one or more labelled substances which are rapidly released and transferred to the perivisceral fluid by passive or active diffusion across the wall of the digestive tube. Since the quantity of algal material fed is restricted, the quantity of these diffusing substances is also limited. An initial peak is to be expected, and when the labelled substances are absorbed by the tissues, a steady-state is attained. In the steady-state, nutrients are mobilized from the reserves at the same rate as they are consumed by the tissues, or the steady-state is maintained due to some control mechanism.

These two hypotheses were tested by a series of experiments. Animals were

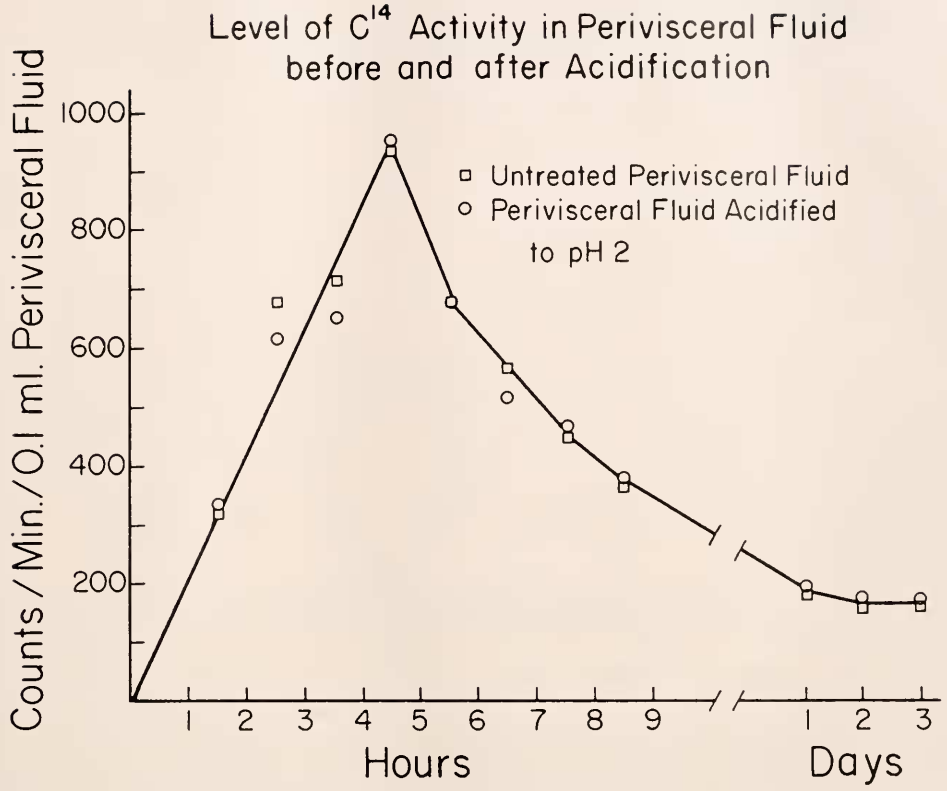

FIGURE 6.

fed labelled algae in the usual manner and the activity in the perivisceral fluid was determined. On the second day after establishment of the steady-state, the animals were fed again and the level of activity in the perivisceral fluid was again followed. Figure 7 shows the results of one of these experiments. If the first hypothesis were correct, a second peak would not have been expected. The first meal would have been expected to correct any nutrient deficiency of the tissues. Since a second peak was observed, the starvation preceding feeding was not responsible for the peak. The second hypothesis would appear to be more tenable, with the peak level representing material which rapidly diffuses from the gut into the perivisceral fluid. The possible involvement of a control mechanism in maintenance of the steady-state cannot be determined by these experiments and will require further study.

The role played by the coelomocytes in the transport of nutrients was determined by measuring the partition of activity between the plasma and cells of the perivisceral fluid at various intervals after the start of feeding. In order to achieve proper geometry for counting and to obtain comparable data, both the cells and the plasma were digested after separation and appropriate dilutions of the digests were pipetted onto the planchets for counting. Figure 8 presents the results and indicates that during the peak of the activity nearly all of the activity is in the plasma phase. Within one day from the start of feeding, as the steady-state is approached,

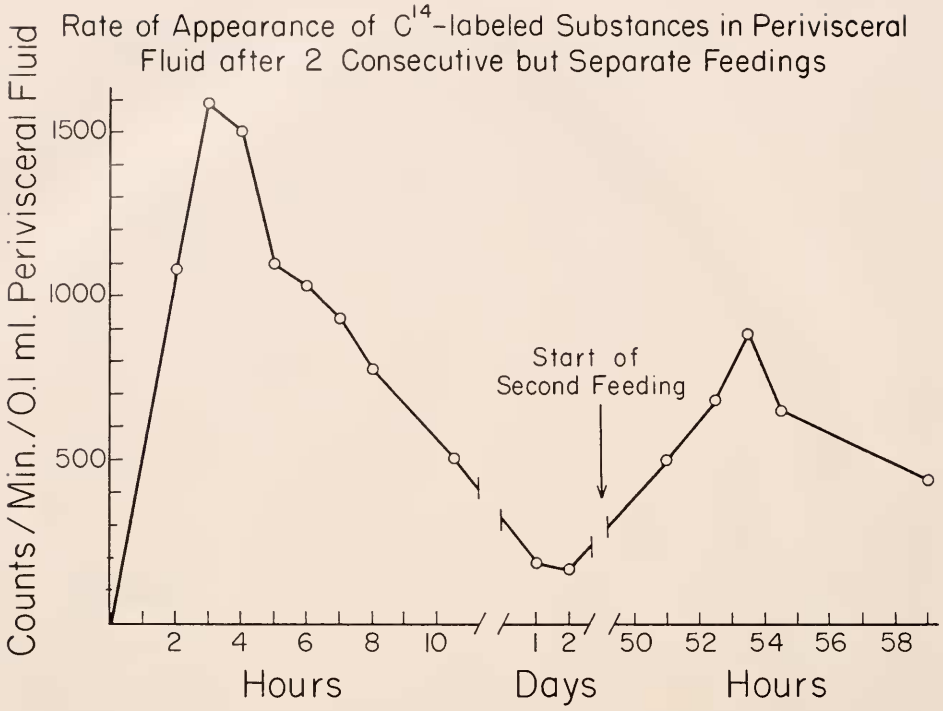

FIGURE 7.

the coelomocytes become the more heavily labelled phase. Table VI presents a set of similar results obtained with additional animals. At the peak level of activity more than 90% of the label is in the plasma. When the activity in the perivisceral fluid levels off, less than 50% of the activity remains in the plasma. Since coelomocytes, particularly the red eleocytes (Boolootian and Giese, 1958) may be observed in the wall of the digestive tube and other tissues, the presence of label in these cells adds support to the view that normal transport of nutrients from the site of reserves to other tissues may be partially achieved via the agency of coelomocytes. The above results do not, however, preclude the possibility that the labelled material in

TABLE VI

Distribution of C^{14}-labelled substances in the plasma and the coelomocytes of the perivisceral fluid of four sea urchins

Animal	Sampled at	Counts/Min/0.1 ml. of		% Activity in plasma
		Perivisceral fluid	Plasma	
A	Peak of activity	843	763	90.5
B	Peak of activity	1073	1070	99.5
C	"Leveled-off" activity	186	36	19.3
D	"Leveled-off" activity	250	116	46.5

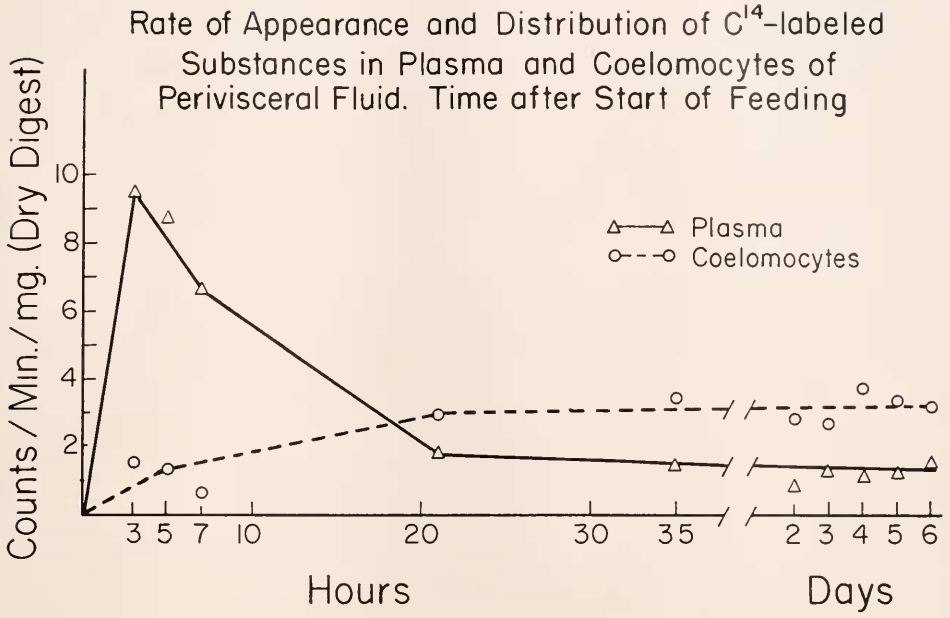

FIGURE 8.

these cells may be attributed to their own nutritional requirements; their appearance in the tissues of the animal may serve other functions.

Attempts were made to identify the labelled compounds found in the perivisceral fluid. Nearly 90% of the activity observed during the peak period was accounted for by one substance which was identified as galactose by its chromatographic behavior in three different solvent systems, conversion to mucic acid, and oxidation by galactose dehydrogenase (Block *et al.*, 1958; Doudoroff, personal communication). Free galactose is not a major constitutent of *Iridaea* tissue. The form of galactose most heavily labelled under the conditions of labelling used here is galactosylglycerol. None of this material could be detected in the perivisceral fluid. This galactoside is apparently hydrolysed by enzymes of the urchin gut.

After the peak level of activity is replaced by the establishment of a steady-state, the C^{14} is distributed among several compounds. Both carbohydrates and amino acids possess activity. Because the level of activity is very low, final identification of these compounds will require microtechniques which have not yet been attempted.

SUMMARY

1. In the purple sea urchin the digestion and absorption of the C^{14}-labelled constituents of the alga, *Iridaea*, occur mainly in the esophagus and adjacent festoons of the stomach.

2. The fauna and flora of the sea urchin gut do not appear to be involved in this digestive process.

3. The absorbed materials are stored mainly in the wall of the gut.

4. During absorption there is a diffusion of labelled material into the plasma of

the perivisceral fluid. A peak level is reached around the sixth hour after the start of feeding. Galactose accounts for 90% of this material and must have been liberated enzymatically from galactose-containing compounds such as galactosylglycerol.

5. The peak level of activity is replaced by a prolonged interval in which the level of activity is reduced but quite constant. The radioactivity is distributed over a variety of compounds including both amino acid and carbohydrates.

6. Translocation of nutrients is accomplished by the perivisceral fluid. No evidence for the participation of either the haemal or water vascular systems could be demonstrated.

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